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Acclimation response of spring wheat in a free-air CO$_2$ enrichment (FACE) atmosphere with variable soil nitrogen regimes. 1. Leaf position and phenology determine acclimation response

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Abstract

We have examined the photosynthetic acclimation of wheat leaves grown at an elevated CO$_2$ concentration, and ample and limiting N supplies, within a field experiment using free-air CO$_2$ enrichment (FACE). To understand how leaf age and developmental stage affected any acclimation response, measurements were made on a vertical profile of leaves every week from tillering until maturity. The response of assimilation (A) to internal CO$_2$ concentration (C$_i$) was used to estimate the *in vivo* carboxylation capacity (*V*c$_{max}$) and maximum rate of ribulose-1,5-bisphosphate limited photosynthesis (*A*$_{sat}$). The total activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), and leaf content of Rubisco and the Light Harvesting Chlorophyll *a/b* protein associated with Photosystem II (LHC II), were determined. Elevated CO$_2$ did not alter *V*c$_{max}$ in the flag leaf at either low or high N. In the older shaded leaves lower in the canopy, acclimatory decline in *V*c$_{max}$ and *A*$_{sat}$ was observed, and was found to correlate with reduced Rubisco activity and content. The dependency of acclimation on N supply was different at each developmental stage. With adequate N supply, acclimation to elevated CO$_2$ was also accompanied by an increased LHC II/Rubisco ratio. At low N supply, contents of Rubisco and LHC II were reduced in all leaves, although an increased LHC II/Rubisco ratio under elevated CO$_2$ was still observed. These results underscore the importance of leaf position, leaf age and crop developmental stage in understanding the acclimation of photosynthesis to elevated CO$_2$ and nutrient stress.

Abbreviations: A$_{sat}$ – CO$_2$ and light saturated rate of CO$_2$-uptake per unit leaf area; CL – control CO$_2$ and limiting nitrogen supply; FL – elevated CO$_2$ and limiting nitrogen supply; CH – control CO$_2$ and high nitrogen supply; FH – elevated CO$_2$ and high nitrogen supply; IRGA – Infra-red gas analyzer; LHC II – light harvesting chlorophyll *a/b* protein primarily associated with Photosystem II; RuBP – ribulose-1,5-bisphosphate; *V*c$_{max}$ – maximum ribulose-1,5-bisphosphate saturated rate of carboxylation *in vivo*

Introduction

The concentration of atmospheric CO$_2$ is expected to double during the 21st century (McElroy 1994). CO$_2$ concentration is limiting for C$_3$ photosynthesis, and so it is generally expected that a rise in atmospheric CO$_2$ concentration will stimulate photosynthesis and increase dry matter production (Kramer 1981). This has been demonstrated in a wide range of C$_3$ plants (Kimball 1983; Gifford 1988; Ziska et al. 1991). Increased

CO_2 uptake in C_3 photosynthesis is primarily due to the fact that the increased substrate concentration at the active site of the primary carboxylating enzyme, Rubisco, suppresses photorespiration (Bowes 1996; Drake et al. 1997). Prolonged exposure to elevated CO_2, especially under nutrient limiting conditions, may result in an acclimatory response of C_3 photosynthesis (Kriedemann and Wong 1984; Sage et al. 1989). Such an acclimatory response may involve a decrease in the amount and activity of Rubisco (Wong 1979; Sage et al. 1989, 1994; Oosten et al. 1992; McKee and Woodward 1994; McKee et al. 1995). Rubisco can account for as much as 50% of the leaf soluble protein and 10–25% of leaf N and is in excess of the level required for photosynthesis when N supply is adequate (Theobald et al. 1998). A decrease in the amount and activity of Rubisco has been observed in wheat grown at elevated CO_2. With adequate N supply, this decrease does not always completely nullify the increased photosynthesis rate due to elevated CO_2. When N supply is limiting, it is possible that Rubisco content will be reduced to a level that will lower light saturated photosynthesis, A, and A_{max} (Evans and Farquhar 1991; Webber et al. 1994). However, results of experiments studying the interactions of CO_2 enrichment and N nutrition have been inconclusive.

While several studies have reported a decrease in leaf Rubisco content with growth at elevated CO_2, others have found no significant loss of this protein, or only a decrease in its activation (reviewed by Long and Drake 1992; Sage 1994; Webber et al. 1994). Wong (1979) showed, in a short-term study with cotton, that down-regulation because of elevated CO_2 was larger at low N-supply than at higher N-supply. Oberbauer et al. (1986) also found in a 3-month study that CO_2 enrichment of *Ledum* resulted in a larger down-regulation in plants grown with lower N supply. On the other hand, there was no evidence for down-regulation in another short-term study on cotton by Wong (1990), or in a short-term study of *Phaseolus vulgaris* grown at high- and low-N supply (Radoglou et al. 1992). Although part of this variation may result from an artificial limitation of rooting volume (Arp 1991), variation in decrease in Rubisco content was still evident among studies in which care was taken to provide a large rooting volume (Long and Drake 1992). Even within the same species, for example wheat, some studies have reported, or inferred, a decrease in Rubisco with growth in elevated CO_2 concentrations (McKee and Woodward 1994) whilst others have not (Delgado et al. 1993; Farage et al. 1998). In a long-term glasshouse study of winter wheat grown at two levels of CO_2 and two levels of N encompassing the full growing season, no evidence of down-regulation was found (Mitchell et al. 1993), even though the plants were grown in pots. This may be attributed to the ability of cereal crops to respond to increased assimilate supply during the vegetative stage by increasing tillering, and during the reproductive phase by translocating more assimilate to the ear (Petterson and McDonald 1994)

Growth of plants under Free-air CO_2 enrichment (FACE) allows a direct study of the effect of elevated CO_2 under field conditions without disturbances frequently encountered within controlled environment and open-top chambers (Hendrey et al. 1993; McLeod and Long 1999). Additionally, FACE studies allow the effects of elevated CO_2 to be monitored continuously throughout a growing season. In an earlier study, spring wheat growing in FACE and well-watered conditions showed reductions in carboxylation efficiency and Rubisco content due to CO_2 enrichment in the top three fully-expanded leaves (Nie et al. 1995; Osborne et al. 1998). Comparing measurements of different growth stages made in separate years, photosynthetic acclimation was observed at the grain filling stage, and was more pronounced in lower leaves when compared to the uppermost fully expanded leaf (Osborne et al. 1998). However, interactions of CO_2 enrichment and nutrient stress were not addressed.

Based upon the previous discussion, there are a number of factors that may lead to an acclimation response to elevated CO_2 in the field. In particular, periods of source sink imbalance, especially when reproductive sinks are developing, may be important determinants for acclimation. Therefore, it is critical that the interaction between leaf age, crop phenology and photosynthetic acclimation in response to growth in elevated CO_2 is studied in more detail. We have tested the hypothesis that leaf age, phenology and N-supply will be important in determining the response of a wheat crop to elevated CO_2 by studying the interaction of CO_2 enrichment and two levels of N nutrition on acclimation of photosynthesis in a field-grown spring wheat crop, comparing the upper-most fully expanded leaf and lower canopy leaves, at each successive developmental stage from tillering through grainfill.

Materials and methods

An experiment was conducted in 1997 to determine in-

teractive effects of elevated CO_2 and limited soil nitrogen on spring wheat (*Triticum aestivum* L. cv. Yecora Rojo) at the University of Arizona Maricopa Agricultural Center (MAC), Maricopa, Arizona, USA. The free-air CO_2 enrichment (FACE) technique was used to enrich the air in circular plots within a wheat field similar to prior experiments (Hendrey et al. 1993; Wall and Kimball, 1993; Dugas and Pinter 1994; Wechsung et al. 1995; Kimball et al. 1995, 1999). For this experiment, the FACE plots were enriched by 200 μmol/mol above ambient ($\sim$ 360 μmol/mol). Unlike the prior experiments, air blowers were installed in the non-CO_2 enriched ambient control plots to provide similar air movement to the FACE plots (Kimball et al. 1999). The FACE treatment was applied continuously from leaf emergence to grain harvest. Each of the circular plots was split into semicircular halves, with each half receiving either an ample (350 kg ha^{-1} season^{-1}) or a limiting (15 kg ha^{-1} season^{-1}) level of nitrogen fertilizer.

Gas-exchange measurements

Measurements were made on leaf material obtained from an undisturbed portion of the canopy. Plant material was sampled prior to 7:30 am to avoid any effects of photoinhibition or water deficit on the leaves. Plants were placed in sealed plastic bags and stored at 10 °C in darkness until measurements were made, according to procedures described by Osborne et al. (1998). The leaf to be measured was excised at the ligule under water and placed in the cuvette of a LI-6400 (LiCor, Inc. Lincoln, Nebraska) with the [CO_2] set at the growth [CO_2]. The leaf was positioned in the chamber at an equal distance from the tip and the ligule. The chamber was illuminated at 1200 μmol photons m^{-2} s^{-1} and the leaf temperature maintained at 20 $\pm$ 0.1 °C. Relative humidity was maintained at 50%. The LI-6400 software/graphics package was used to determine whether steady-state photosynthesis had been reached. When steady-state photosynthesis was reached, the program to vary intercellular [CO_2] (C_i) was used to measure photosynthesis at C_i's ranging from 50 to 1000 μmol CO_2 mol^{-1}. The initial linear slope of the A/C_i response was used to estimate Vc_{max} according to the method of Wullschleger (1993) using the parameter coefficients of Harley et al. (1992). A_{sat} was measured at 1000 μmol CO_2 mol^{-1}. At the end of the A/C_i measurement program, the leaf was allowed to reach steady state photosynthesis at its growth [CO_2]. When steady-state photosyn-

thesis was reached, the leaf was removed from the cuvette and immediately freeze-clamped with a liquid nitrogen-cooled clamp, and stored in liquid nitrogen until biochemical assays were conducted.

Biochemical assays

Leaf tissue was removed from liquid nitrogen and ground in an ice-cold glass homogenizer containing 100 mM Tricine (pH 8.0), 5 mM $MgCl_2$, 0.1 mM EDTA, 5 mM DTT, 1% (w/v) PVP, 0.05% (v/v) Triton X-100 and 1 mM phenylmethylsulfonyl fluoride at a ratio of 1 cm^2 leaf tissue to 1 ml buffer. An aliquot was assayed for full activity and *in situ* activity of Rubisco following the assay procedure of Salvucci and Anderson (1987), with the exception that casein was not included in the assay mixture. Aliquots were saved for chlorophyll and total soluble protein determination and stored at –20 °C. Chlorophyll concentrations were determined according to Arnon (1949). Additional aliquots were boiled in SDS sample buffer and used for SDS–PAGE as described by Nie et al. (1995). Following SDS–PAGE, the gels were stained with coomassie blue and the relative intensity of each band quantitated using a laser scanning densitometer. The relative amount of Rubisco and LHC II was determined from the area under the corresponding peaks as described previously (Nie et al. 1995).

Statistical analysis

The effect of nitrogen on photosynthetic acclimation to elevated CO_2 was examined using a two way ANOVA for A_{sat}, Vc_{max} and Rubisco activity. Gas exchange measurements and biochemical assays were replicated three times for each sample treatment.

Results

The A/C_i response of individual leaves of FACE grown spring wheat was measured throughout the growing season for the uppermost, fully expanded leaf (flag leaf) and next two successively older leaves (flag-1 and flag-2). Material was harvested from each leaf for biochemical analysis (see 'Materials and methods'). The results describe first a statistical analysis of the overall effect of elevated CO_2 and N supply on photosynthetic parameters. This is followed by a more detailed analysis of these parameters for each leaf at different growth stages, to understand

Table 1. Overall analysis of season long pooled data for each leaf showing the effects of ambient and high CO_2 at both N levels

Leaf		Low CO_2	High CO_2[a]	$P > F$	Low N	High N	$P > F$	Low N		High N		Interaction
								Low CO_2	High CO_2[b]	LowCO_2	High CO_2[b]	$P > F$
Vcmax (μmol CO_2 m^{-2} s^{-1})	Flag	44.8	42.1 (6)[b]	0.0393	41.1 (10.3)	45.8	0.0020	43.6	38.6 (11.5)	46.05	45.6 (1.0)	0.1209
	Flag-1	41.4	30.3 (26.8)	0.0001	31.8 (20.3)	39.9	0.0373	35.7	27.9 (21.8)	47.1	32.7 (30.6)	0.0997
	Flag-2	29.7	23 (22.6)	0.1783	21.4 (31.6)	31.3	0.0001	24.2	18.5 (23.6)	35.2	27.4 (22.2)	0.5269
A_{sat} (μmol CO_2 m^{-2} s^{-1})	Flag	27.4	26.5 (3.3)	0.3709	25.7 (8.9)	28.2	0.1339	26.2	25.2 (3.8)	28.6	27.9 (2.4)	0.8234
	Flag-1	22.1	19.2 (13.1)	0.0792	18.6 (18.1)	22.7	0.0662	19.5	17.8 (8.7)	24.6	20.7 (15.9)	0.3677
	Flag-2	15	12.4 (17.3)	0.0116	11.5 (27.7)	15.9	0.0001	12.4	10.6 (14.5)	17.6	14.2 (19.3)	0.4356
Total Chl. (mg Chl cm^{-2})	Flag	41.2	40 (2.9)	0.1666	32.3 (33.9)	48.9	0.0158	32.9	31.6 (4.0)	49.62	48.3 (2.6)	0.9842
	Flag-1	41.6	39.2 (5.8)	0.2715	30.2 (40.3)	50.6	0.0017	31.1	29.3 (5.8)	52	49.1 (5.6)	0.6773
	Flag-2	33.1	30.8 (6.9)	0.2776	22.1 (47.1)	41.8	0.0024	23.5	20.8 (11.5)	42.7	40.9 (4.2)	0.8237
Rubisco (μmol CO_2 m^{-2} s^{-1})	Flag	75.6	66 (12.7)	0.0001	54.7 (37.1)	86.9	0.0012	59.3	50.2 (15.3)	92	81.8 (11.1)	0.8029
	Flag-1	59	44.6 (24.4)	0.3967	39 (39.6)	64.6	0.0996	45.1	32.9 (27.1)	72.9	56.3 (22.8)	0.5973
	Flag-2	32	21.7 (32.2)	0.0018	19.2 (44.5)	34.6	0.0001	23.1	15.2 (34.2)	41	28.2 (31.2)	0.4419

[a]Value in parentheses denotes percent decrease from ambient CO_2.
[b]Value in parentheses denotes percent decrease from high N.

how any response was influenced by leaf position and developmental age of the crop.

Overall treatment effect

Table 1 summarizes an overall analysis, by leaf, of season-long pooled data. A two-way ANOVA indicates a decrease in Vc_{max} and A_{sat} due to CO_2 enrichment or N stress for the flag, flag-1 and flag-2 leaf. This decrease in Vc_{max} and A_{sat} was more pronounced for leaves lower in the canopy, indicating a significant influence of leaf age and position on the parameters measured. Significant reduction in total chlorophyll and total Rubisco activity due to the N stress or CO_2 enrichment were also observed, again with the greatest reduction occurring in older leaves (Table 1). CO_2 enrichment resulted in greater reduction at low N than at high N for all parameters in the flag leaf (Table 1). The flag-1 leaf showed greater reduction in Vc_{max} and A_{sat} due to CO_2 enrichment under high N than under low N. Total chlorophyll and Rubisco activity showed larger reductions under low N in the flag-1 leaf. Reduction due to CO_2 enrichment for the flag-2 leaf was greater under low N for Vc_{max}, total chlorophyll and Rubisco activity, and greater under high N for A_{sat}.

Analysis by developmental stage

Photosynthetic gas exchange parameters were measured every week from tillering, which commenced 72 days after planting (DAP 72), until maturity at DAP 143. Results from this analysis are presented in Figures 1 and 2. Prior to canopy closure at the boot stage, only the uppermost fully expanded leaf was measured. Photosynthetic gas exchange parameters (Figures 1 and 2) and Rubisco activity (Figure 3) progressively decreased with leaf position down the stem. Comparing leaves grown at ambient CO_2 and high N, A_{sat} and Vc_{max} decreased by approx 25% in the flag-2 leaf compared to the flag leaf at the heading stage. By the late milk dough stage, this reduction was 45%. Rubisco activity (Figure 3) was reduced 50% in the flag-2 leaf at the late milk dough stage. At the heading stage, the chlorophyll *a/b* ratio (not shown) was reduced from approximately 3.2 in the flag leaf, to 2.7 in the flag-2 leaf, consistent with a slight increase in content of the chlorophyll *a/b*-containing light harvesting complexes in response to the lower light level deeper in the canopy. At the late milkdough stage, the chlorophyll *a/b* ratio was 2.9 in the flag leaf and 2.5 in the flag-2 leaf.

Flag leaf

A gradual decline in A_{sat} was observed from an average value of 40 μmol m^{-2} s^{-1} at tillering to approx 17 μmol m^{-2} s^{-1} at the soft dough stage (Figure 1). Neither elevated CO_2 nor N-stress had any pronounced effect on A_{sat} at any growth stage. A similar progressive decline in Vc_{max} was observed during the growing season (Figure 2). Significant $CO_2 \times N$ interactions on Vc_{max} were not observed at any growth stage. Rubisco full activity (Figure 3) was reduced by N-stress, ranging from an approximately 23% reduction at stem extension, to a 46% reduction by the end of anthesis. Significant $CO_2 \times N$ interactions for Rubisco activity were found at several different growth stages. At tillering, milkdough stage (DAP122) and late milkdough stage (DAP 126) Rubisco activity was reduced 21% ($P = 0.0287$), 46% ($P = 0.0192$), 35% ($P = 0.2774$), respectively, by CO_2 enrichment and N-stress. At late anthesis (DAP 117), Rubisco activity was reduced 25% ($P = 0.3244$) by CO_2 enrichment under low N, and 36% ($P = 0.0908$) under high N. Total chlorophyll content (Figure 4) was reduced 47% by N-stress ($P = 0.0155$) at mid-anthesis, and a similar N-stress reduction in total chlorophyll was observed the remainder of the growing season.

Flag-1

A_{sat} (Figure 1) was reduced 33% by CO_2 enrichment at both low ($P = 0.0165$) and high N ($P = 0.0109$) at the heading stage (DAP 99). At the early milk-dough stage (DAP 122), CO_2 enrichment reduced A_{sat} (41%, $P = 0.0042$) at both N levels. Significant $CO_2 \times N$ interactions in A_{sat} were not observed. The highest rates of Vc_{max} (Figure 2) and Rubisco activity (Figure 3) were observed for plants grown at atmospheric CO_2 and high N. Largest reductions in Vc_{max} due to elevated CO_2 were observed under high N at early anthesis, mid-anthesis and soft dough stage. $CO_2 \times N$ interactions were also observed in Rubisco activity during boot stage (DAP 92) when elevated CO_2 resulted in a 28% reduction in Rubisco activity ($P = 0.0581$) under low N, but had no effect under high N (Figure 3). From mid-anthesis onward, $CO_2 \times N$ interactions in Rubisco activity were due to reduced activity at elevated CO_2 and high N (reductions of 36%, 34% and 18% at DAP 112, 117 and 126, respectively). By the heading stage, N-stress had a clear effect on total chlorophyll content (Figure 4). During early anthesis (DAP 108) and onwards, total chlorophyll was re-
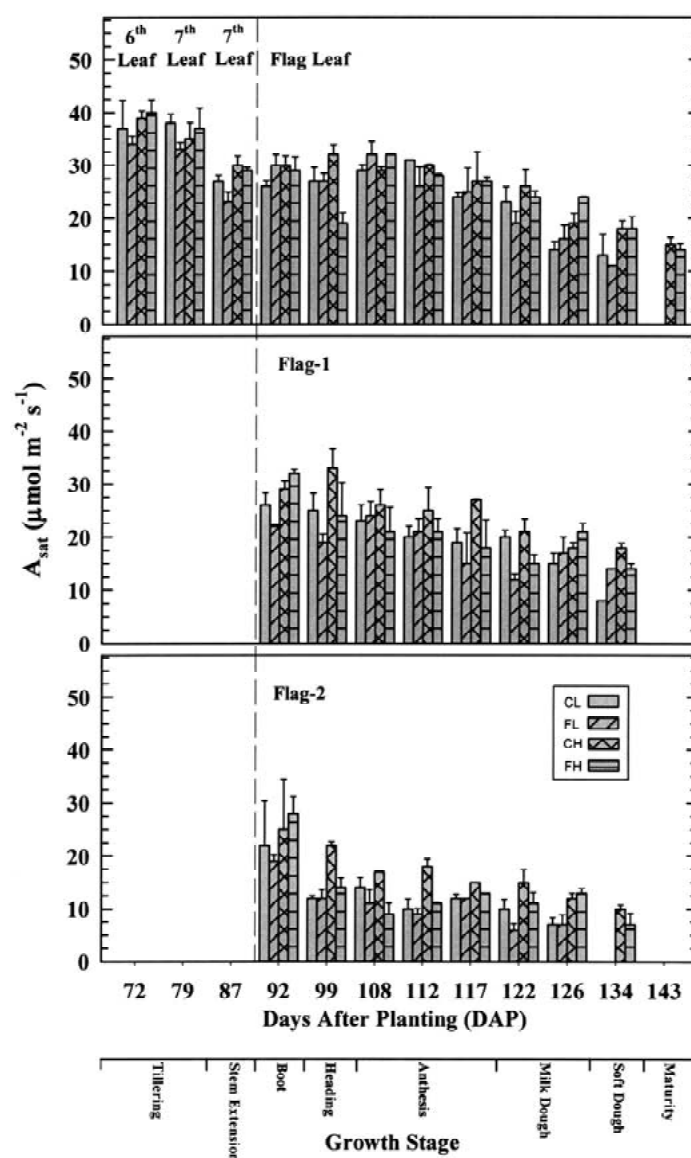
Figure 1. A_{sat} for the flag, flag-1 and flag-2 leaves in wheat grown in elevated CO_2 and in control CO_2 conditions with ample and limiting N supplies, measured at different developmental stages. The days after planting (DAP) and growth stage, determined by Zadok's scale, are indicated beneath the figure. CL – control CO_2 and limiting nitrogen supply; FL – elevated CO_2 and limiting nitrogen supply; CH – control CO_2 and high nitrogen supply; FH – elevated CO_2 and high nitrogen supply.

duced by 40–48% by N stress. No significant changes were observed in the chlorophyll *a/b* ratio.

Flag-2

Significant $CO_2 \times N$ interactions were found in A_{sat} for the flag-2 leaf at the heading, early and mid-anthesis stages, and milkdough stages (Figure 1). At heading, early and mid-anthesis, CO_2 enrichment at high N reduced Asat by 29%, 42% and 47%, re-

spectively. At the early milk dough stage, A_{sat} was decreased by 40% at low N and 27% at high N. The highest rates of Rubisco activity and Vc_{max} were observed for the plants grown at atmospheric CO_2 and high N, similar to that observed for the flag-1 leaf. Vc_{max} was reduced (44%) by N-stress at both control and elevated CO_2 levels at early anthesis and early milk-dough stages (Figure 2). At heading, CO_2 enrichment reduced Vc_{max} at high N (33%; $P = 0.0186$).
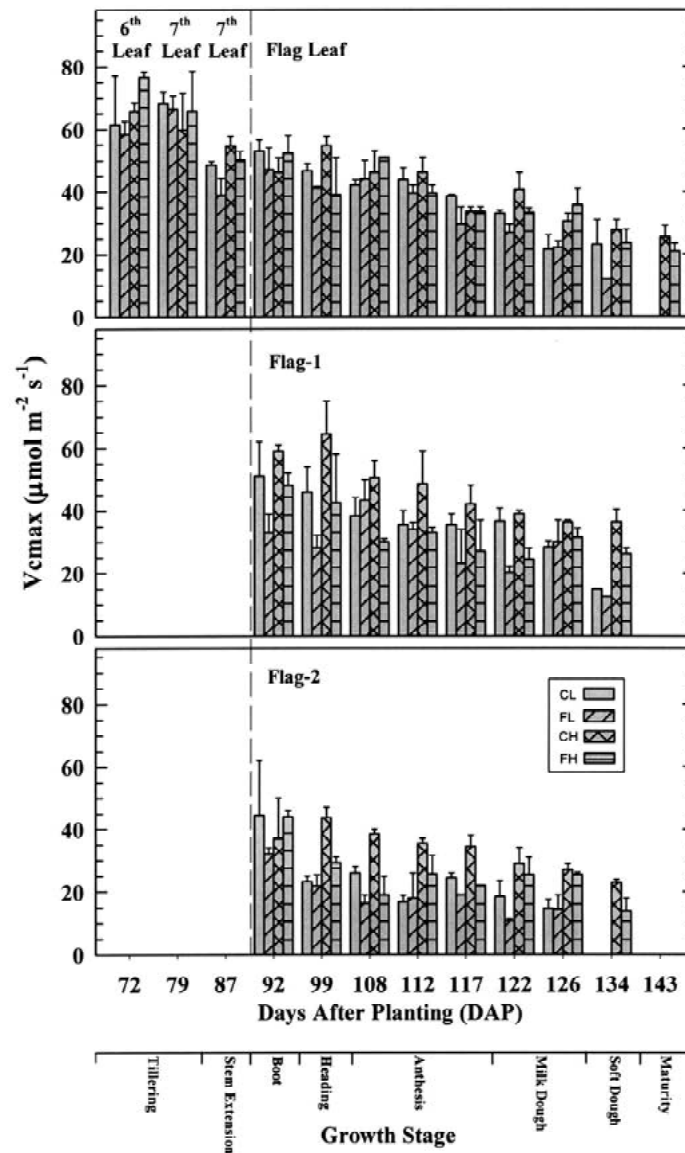
Figure 2. Vc_{max} for the flag, flag-1 and flag-2 leaves in wheat grown in elevated CO_2 and in control CO_2 conditions with ample and limiting N supplies, measured at different developmental stages (Figure 1).

At late anthesis, Vc_{max} was reduced 22% ($P = 0.1477$) by CO_2 enrichment at low N, and 36% at high N ($P = 0.0110$). At the early milk dough stage, Vc_{max} was reduced more at low N (42%) than at high N (12%).

Rubisco activity (Figure 3) showed significant $CO_2 \times N$ interactions at many growth stages. At the boot stage, elevated CO_2 caused a 43% reduction in Rubisco activity at low N ($P = 0.3729$). At the three anthesis stages, CO_2 enrichment at high N reduced Rubisco activity by an average of 55%. At the early milk dough stage, this pattern reversed, and Rubisco activity was reduced 63% at low N and 27% at high N.

At the heading stage, N-stress reduced total chlorophyll (45%, $P = 0.0177$; Figure 4). The N-stressed reduction in total chlorophyll progressively increased at subsequent growth stages. By late milk dough stage, total chlorophyll was decreased 69% ($P = 0.0424$) by N stress. The chlorophyll *a/b* ratio of 2.5 did not significantly change at any of the different growth stages (not shown).
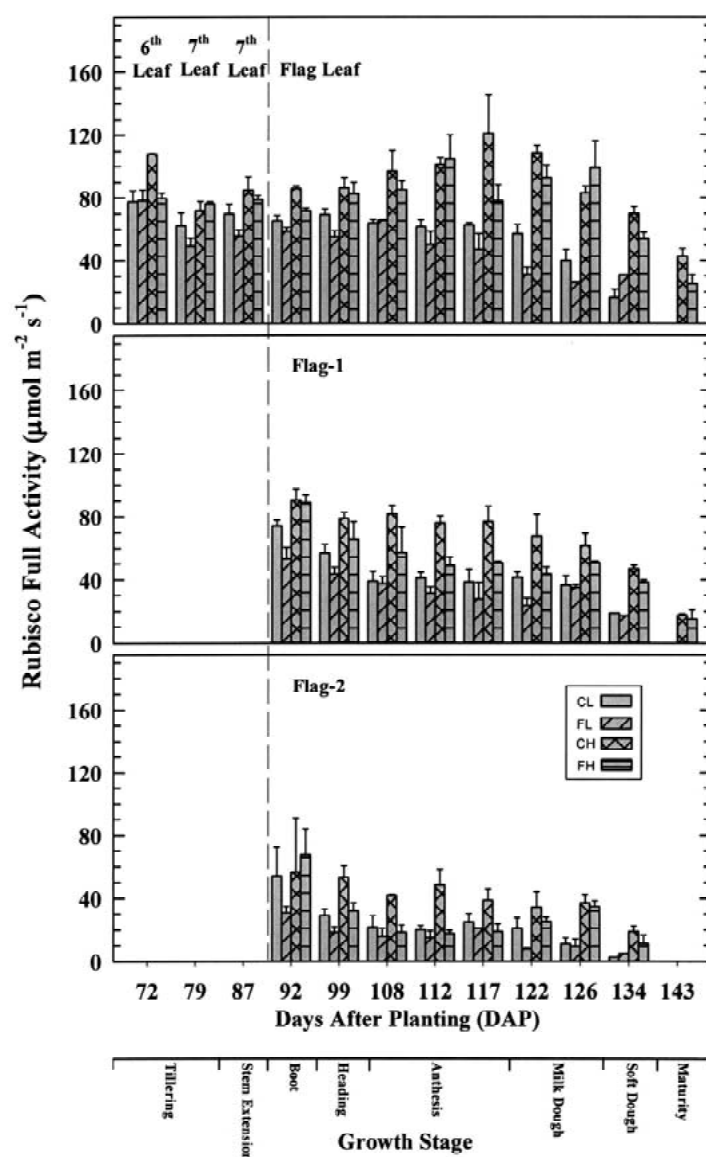
Figure 3. Rubisco full activity for the flag, flag-1 and flag-2 leaves in wheat grown in elevated CO_2 and in control CO_2 conditions with ample and limiting N supplies, measured at different developmental stages (Figure 1). Leaf material was sampled from the same portion of the leaf for which A_{sat} and Vc_{max} were determined.

Protein content

At selected growth stages, total protein was isolated from leaf material and size fractionated by SDS–PAGE (Figures 5 and 6). Rubisco content of the flag-2 leaf, at normal N, was reduced 40–50% compared to the flag leaf. Changes in the accumulation of Rubisco protein were found to correlate closely with the observed changes in total Rubisco activity. This indicated that the decreased Vc_{max} measured *in situ* was due

to reduced Rubisco activity, resulting from a lowered Rubisco content per leaf surface area. LHC II content under control conditions (high N and atmospheric CO_2 level) increased 25% from DAP 92 to DAP 126 (Figures 5 and 6). The flag-1 and flag-2 leaves had a 30–50% higher LHC II content at DAP 122 and 126 compared with the flag leaf at DAP 92. The net result of changes in LHC II – Rubisco content was an increase in the ratio of LHC II to Rubisco in the older leaves, relative to the flag leaf. Elevated CO_2 also in-
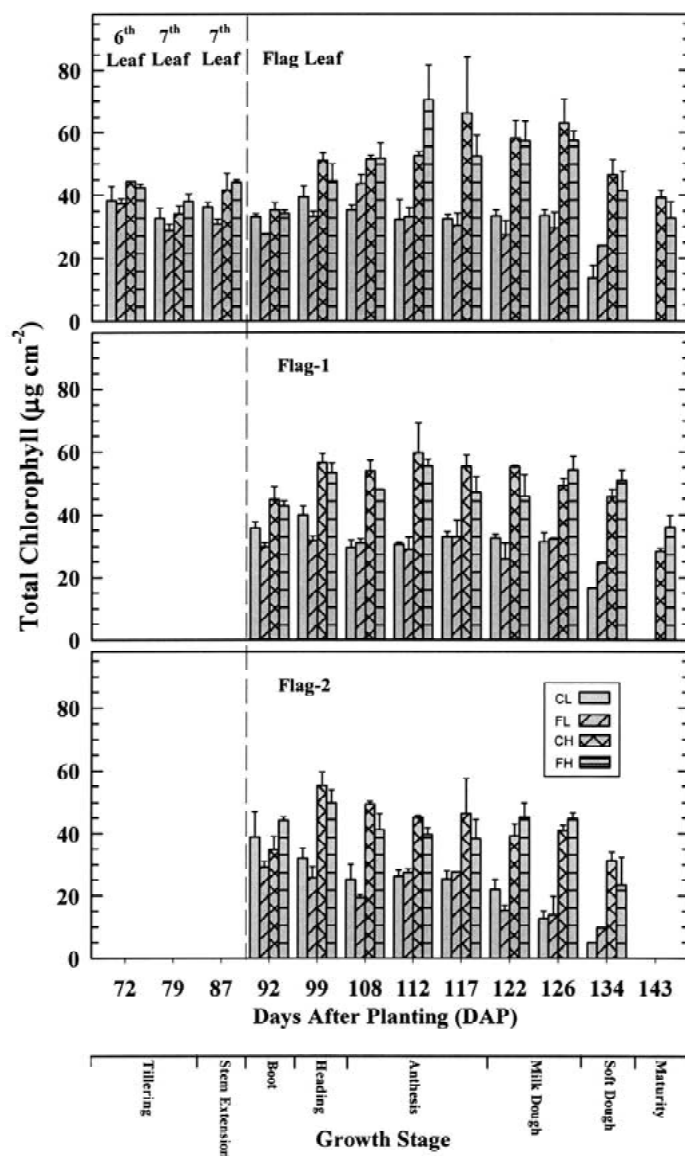
Figure 4. Total chlorophyll content for the flag, flag-1 and flag-2 leaves in wheat grown in elevated CO_2 and in control CO_2 conditions with ample and limiting N supplies, measured at different developmental stages.

creased the ratio of LHC II to Rubisco in the flag-1 and flag-2 leaves under ample N conditions. Under low N, the LHC II content and Rubisco content was reduced 50–75% in the flag-2 leaf, indicating an effect of N stress on the accumulation of these two proteins. Nevertheless, the LHC II–Rubisco ratio still increased in the older leaves under the low N conditions, although the effect of elevated CO_2 was less.

Discussion

The acclimatory response of individual leaves to growth under N-stress and in elevated CO_2 was strongly dependent upon leaf position in the canopy and upon the developmental stage of the crop. A progressive decline in Vc_{max} was observed with age of the flag leaf, but there was no significant acclimatory response to elevated CO_2 at either N-level. The flag-1 and flag-2 leaves showed a significant acclimation of Vc_{max} in response to growth in elevated CO_2. De-
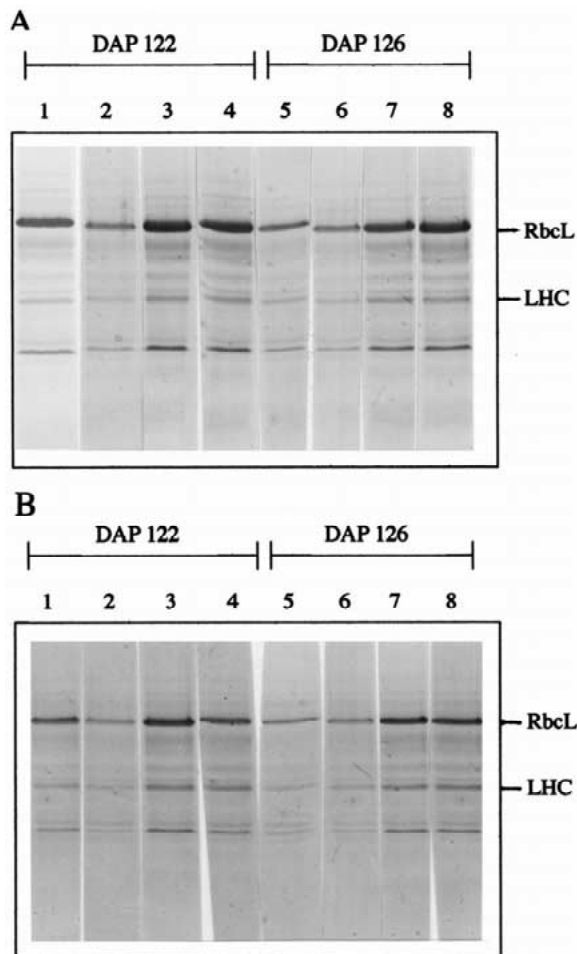
Figure 5. Protein profile of the flag (A) and flag-2 (B) leaves of wheat grown in elevated CO_2 and in control CO_2 conditions with ample and limiting N supplies, measured at different developmental stages (DAP 122 and DAP 126). Protein isolates were size fractionated by SDS–PAGE, and polypeptides visualized by staining with Coomassie blue. Lanes 1 and 5, CL; 2 and 6, FL; 3 and 7, CH; 4 and 8, FH.

pending on the developmental stage, this acclimation occurred under either N-stress, ample N or under both N levels. Acclimation of Vc_{max} in the lower leaves was apparent at the heading stage through to the early milk dough stage, but was not observed at the late milk dough stage. In an earlier study of FACE-grown wheat, comparing measurements of different growth stages made in separate years, photosynthetic acclimation in lower leaves at the grain filling stage was also observed (Osborne et al. 1998). In this study, a season-long analysis involving measurements at eight different growth stages clearly indicates the importance of leaf position and phenology in determining the

response of individual leaves to elevated CO_2. Furthermore, the interaction of N-supply with growth in elevated CO_2 also is dependent on which leaf is studied and on the developmental age of the crop at the time of measurement.

Rubisco can account for as much as 50% of the leaf soluble protein and is a major N investment of the plant. Rubisco can accumulate in excess of that required for photosynthesis when N supply is adequate. However, when N-supply is limiting, it is expected that Rubisco accumulation will be reduced to a level sufficient for maximum photosynthesis, freeing up N for use elsewhere in the plant (Evans and Farquhar 1991; Webber et al. 1994). Experimental evidence has shown that a decline in Vc_{max} is due to a decreased Rubisco content or activity. Based on the aforementioned considerations, reduction in Vc_{max} with elevated CO_2 is expected to be more pronounced under N-stress growth conditions. In the flag leaf, the progressive decline in Vc_{max} with leaf age was not paralleled by a similar decline in Rubisco activity at high N. Osborne et al. (1998) attributed this to a decreased activation state of Rubisco with leaf age. The reduced Rubisco activity at low N at each developmental stage, however, was matched by a corresponding decrease in Vc_{max}. Neither Vc_{max} nor Rubisco activity in the unshaded flag leaf was significantly altered by elevated CO_2, consistent with earlier FACE studies of wheat (Nie et al., 1995). In lower leaves (flag-1 and flag-2), the decline in Vc_{max} with age and developmental stage correlated with reduced Rubisco activity and content. The decline in Vc_{max} due to elevated CO_2 was more pronounced when N supply was adequate, particularly during anthesis (Figure 2). A decrease in Vc_{max} and Rubisco activity for N-stressed leaves in elevated CO_2 was only observed prior to anthesis and again during late anthesis and early milk dough stages. In lower leaves, elevated CO_2 had little effect on the level of leaf total soluble protein (not shown), especially at low N. This would imply that acclimation observed under these conditions was specific for Rubisco, and was not a dilution effect.

In monocotyledons, such as wheat, new leaves emerge into full sunlight at the top of the canopy and then become more shaded as new leaves emerge above them. The decline in Vc_{max} and A_{sat} with depth in the canopy, therefore, corresponds with the degree of shading and age of the leaves. Typical shade responses of the photosynthetic apparatus include an increase in LHC II proteins and a decrease in Rubisco content (Anderson 1986; Evans 1993, 1996). In this study,
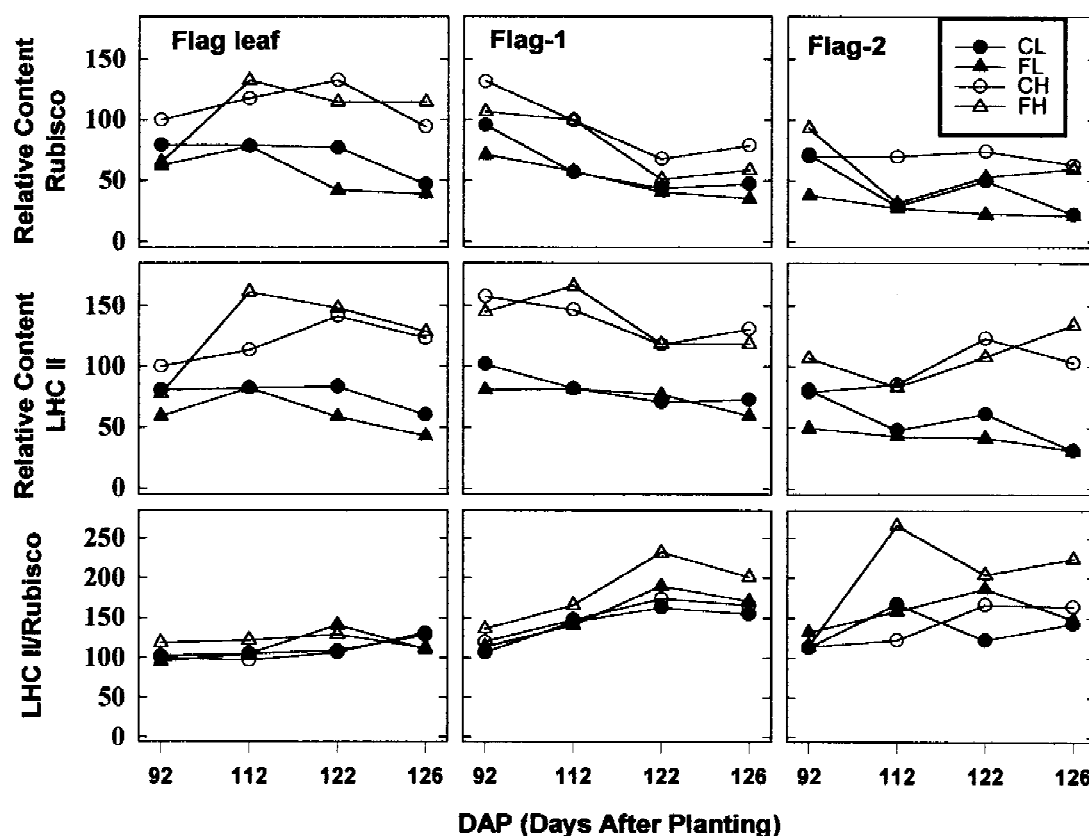
Figure 6. Rubisco, LHC II and LHC II/Rubisco for the flag, flag-1 and flag-2 leaves in wheat grown in elevated CO_2 and in control CO_2 conditions with ample and limiting N supplies, measured at different developmental stages. The relative amount of Rubisco and LHC II was determined by scanning of gels shown in Figure 5 (see 'Materials and methods'). Values are the protein content determined on a leaf area basis, and expressed in arbitrary units relative to the absorption of the corresponding protein in control leaves at DAP 92.

shade adaptation was reflected by the increased LHC II/Rubisco ratio observed in the lower leaves. With ample N, LHC II content was not reduced in the lower leaves, indicating that the increased ratio of LHC II – Rubisco was related to light acclimation and not senescence. Under limiting N supply, the LHC II content was reduced in the flag-2 leaf, suggesting that some senescence may have occurred. Even with limiting N supply, the LHC II – Rubisco ratio increased in the flag-2 leaf indicating light acclimation. Canopy architecture and light penetration, however, were not significantly changed by growth in elevated CO_2 when N supply was adequate. Under low N, canopy architecture was significantly different to that of plants growing at ample N (Brooks et al. 2000). At low N, the canopy was more erectophile, but this resulted in only a slight increase in light penetration to the lower leaves compared to ample N conditions, and was unaffected by elevated CO_2. This was reflected by the

fact that the LHC II – Rubisco ratio in the N-stressed leaves was similar to that of leaves of plants receiving adequate N (Figure 6). Therefore, it is unlikely that the acclimation response to elevated CO_2 observed in the lower leaves was due to increased shading in either the N-stress or ample N treatments.

Several factors may contribute to the different responses of wheat leaves at different developmental stages in elevated CO_2. Changes in resource allocation will occur as the crop switches from vegetative growth to the production of reproductive structures and grain filling. During the developmental sequence, leaves will transition from an initial sink to a source of photosynthate and N. In particular, N tied up in Rubisco is likely to be reallocated to grain development (Simpson et al. 1983; Simpson, 1992), and such a reassignment would explain the more pronounced decline in Vc_{max} and Rubisco activity at elevated CO_2 in leaves lower in the canopy at the late anthesis and

grain filling stage. In addition, growth in elevated CO_2 with ample N could result in increased growth and rate of production of the reproductive structures compared to growth at either CO_2 concentration under N-stress. This increased growth would explain the observed dilution of leaf N content (Sinclair et al. 1998), particularly in leaves of plants grown at low N and elevated CO_2 and lead to an acclimatory decline in Vc_{max} and A_{sat} (Osbourne et al. 1998). In any case, this acclimation response would be expected to be functionally significant because less Rubisco would be required to maintain a constant photosynthetic rate in elevated CO_2 conditions.

Results from *in situ* studies also reported in this issue indicate as much as a 30% increase in carbon gain in the uppermost fully expanded leaves due to elevated Ca (Wall et al. 2000). Some stimulation of C_3 photosynthesis at elevated CO_2 is expected despite the observed reduction in A_{sat} and Vc_{max} because the oxygenation of RuBP is suppressed. This is in agreement with results reported by Osborne et al. (1998), who interpreted this to indicate that acclimation was best interpreted as increased efficiency of resource allocation rather than an adverse reaction to elevated CO_2.

In contrast, it is important to emphasize that the whole-canopy net assimilation rate showed at most only a 10% increase (Brooks et al. 2000). Presumably there are compensatory changes in canopy architecture that minimize the overall effect of the responses observed at the individual leaf level. Indeed, results of above and below ground biomass production were in better agreement with the whole-canopy net assimilation rates than to results obtained from individual leaves.

In conclusion, there is still considerable controversy as to the extent to which photosynthetic apparatus will acclimate in response to future elevated CO_2 atmospheres. This is partly due to the fact that previous work has focused only on the uppermost fully expanded leaf, and reletively few studies (e.g. Osborne et al. 1998) have considered leaf age and crop phenology as a variable. This work demonstrates that acclimation of the photosynthetic apparatus of wheat will occur in response to elevated CO_2. However, the response is most pronounced in lower shaded leaves and at later stages of crop development. Taken together with results reported in other papers in this volume (Brooks et al. 2000; Wall et al. 2000), this work emphasizes the inherent complexities in trying to predict the acclimatory responses of a wheat crop to growth at elevated CO_2.

Acknowledgements

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